Quality Assurance in the Manuela Joint Action (field experiment)

Draft document prepared by participants of the experimental set-up workshop
Sines, Portugal
5 – 9 February 2007

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1 Introduction

The purpose of this document is to standardise the methods used for the Joint Manuela Action (Manuela proposal task 4). Parallel field experiments will be carried out at four geographically separated European beaches to investigate the response of meiofauna to the same type and frequency of disturbance.

This Quality Assurance (QA) document aims to ensure comparability of resultant data and prevent errors in the various stages of the experiments. QA should be considered at every phase from site selection to sample collection and through to data analysis and reporting. ICES recommends that the following topics be included in any QA document:

1. Formal listing of personnel involved.
2. Procedures for the handling and use of chemicals (i.e. formaldehyde and other reagents) in marine environmental surveys.
3. Procedures for handling survey equipment.
4. Procedures for station selection and location, as well as navigational accuracy and documentation.
5. Procedures for the collection of biological material.
6. Procedures for the storage of biological material.
7. Procedures for sorting biological material.*
8. Procedures for the distribution of sorted biological material for taxonomic analysis. Signed protocols should be obligatory for all steps in analysis.
9. Procedures for identifying biological material.*
10. Taxonomic accreditation of the persons at the laboratories should be aimed at. Training should be offered by institutions possessing the appropriate level of expertise in the form of regular exchange of personnel.
11. Procedures for the recording of biological and environmental data.**
12. Procedures for the analysis of biological and environmental data.
13. Procedures for report writing and documentation.
14. Details for the professional qualifications of survey and laboratory personnel.

* These procedures should include random control checks of both sorting and identification by experienced personnel.

** These procedures should include obligatory proof-reading before entering into a computer and before usage.

The experimental design and the timing of the experiment is summarised in the 'Manuela Joint Action Working Plan', circulated by M Steyaert in September 2006. Subsequently, this QA guidance has been produced to ensure that the conduct of the experiment and the processing and analysis of samples can be as accurate and similar as possible between laboratories.

Detailed protocols have been prepared, describing each step of the experiment. These are attached as appendices and should be read before any work is undertaken.

2 Experimental set-up

A field manipulation experiment will be carried out at four geographically separated European beaches, investigating the response of meiofauna communities to increased intensity and frequency of rainfall as predicted by climate change models. The study will specifically test the null-hypothesis:

H_0: Sandy beach meiofauna from different geographic areas respond in comparable ways to the same type, intensity and frequency of disturbance (i.e. increased fresh water input as a result of climate change).

The experimental sites are located at Arina (Crete, Mediterranean Sea: microtidal beach), De Panne (Belgium, North Sea: macrotidal, dissipative beach), Hel (Poland, Baltic Sea: microtidal beach) and Sines (Portugal, Atlantic coast: macrotidal, dissipative beach).

Five blocks with two treatments each are set up along each beach (Figure 2.1). Blocking of treatments should allow the estimation of the effect of any spatial variability across the study area against any treatment effect (Underwood AJ. 1997. Experiments in ecology. Cambridge University Press, Cambridge, UK, 504 pp).

Plots should be located with their centre points along a line (also see Figure 3.1) 1 m above Mean High Water Spring (MHWS).

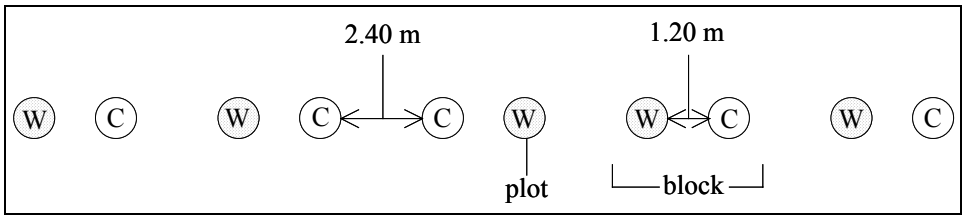


Figure 2.1 Schematic diagram of a randomised block design (C = control, W = treatment). The order of controls and treatments is random and might differ between beaches

Experimental plots are circular with a 2 m diameter. An area of 2.40 m diameter is treated with rainwater to ensure that all samples can be collected away from the plot edge. A central peg with a line attached is used to describe the circumference of each plot (Figure 2.2 and 2.3). Plots within a block are 1.20 m apart, blocks are 2.40 m apart (Figure 2.1).

Figure 2.2 Tracing of experimental plot

Figure 2.3 Experimental block consisting of a control and treatment plot

3 Location of experimental plots

Sediment in the experimental plots is not contained by physical structures (e.g. meshes, cages etc.) to remove caging effects, but this is likely to complicate the identification of each plot for manipulation and sampling.

The centre of each plot is marked with a stake. This should be driven into the sediment as deep as possible, leaving it so that 5 cm is exposed above the sediment surface. On each sampling occasion, plot locations are confirmed using this, marked ropes and reference landmarks (Figure 3.1, 3.2 and 3.3).

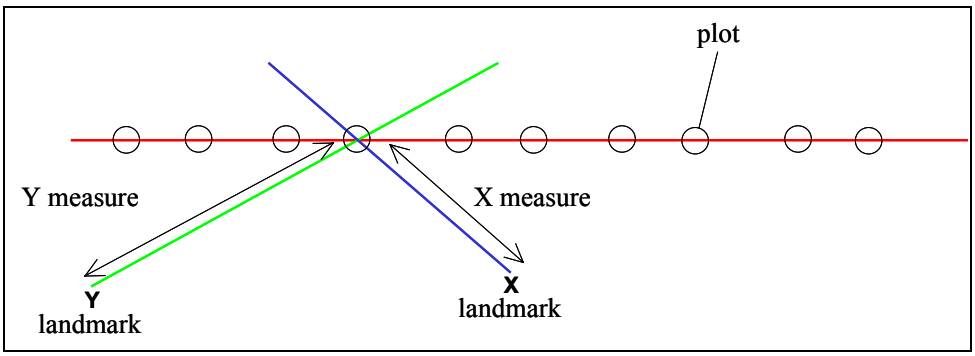


Figure 3.1. Method of plot location. All plots should be parallel to and 1 m above MHWS, following the red rope. The red rope also requires (some) landmark(s)

To locate each plot, the red, green and blue rope is used together with landmark 1 and 2. In the example, all 5 plots can be found with the two landmarks, but, depending on the geography of the beach, more than one pair of landmarks might be required. For each plot, an X and Y measurement is needed.

Figure 3.2. Attachment of rope to landmark

Figure 3.3. Plot location

3.1 Potential sources of errors during the location of experimental plots

Potential errors during the location of experimental plots can occur:

- Identification of landmark
- Measurement of distances

4 Treatment of experimental plots ('rainfall')

Watering cans should be used to apply 80 litres of fresh water to the plots. Water should be applied by two individuals simultaneously: 2 x 10 l should be applied standing at opposite sides of the plot (Figure 4.1 and 4.2). The point of application should move 90 degrees clockwise until all water is applied, so on the second application 2 x 10 l are applied perpendicular to the starting positions (Figure 4.3a) On each day, the starting position for water application should moved clockwise by 45 degrees (Figure 4.3b).

Figure 4.1. Application of fresh water

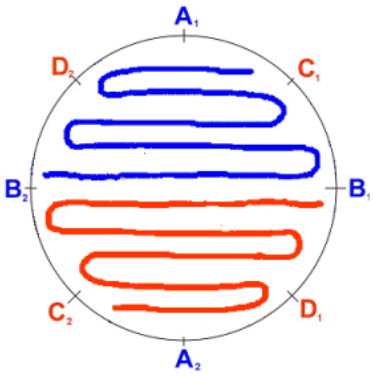


Figure 4.2. Schematic diagram of fresh water application

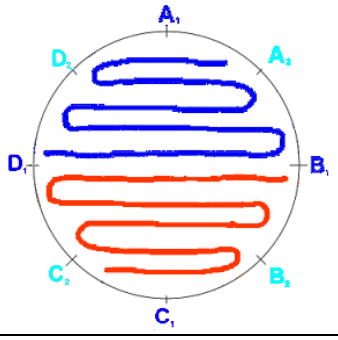
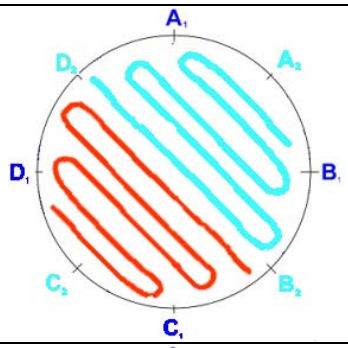
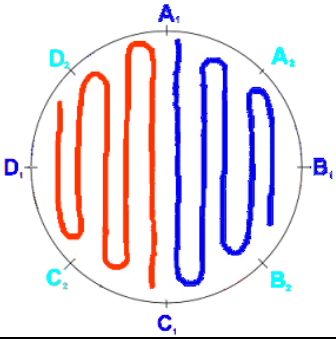
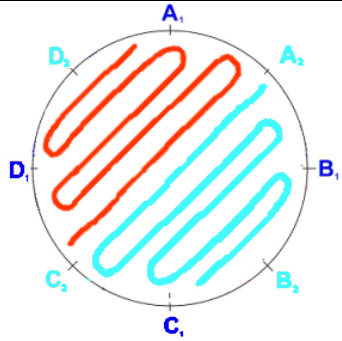
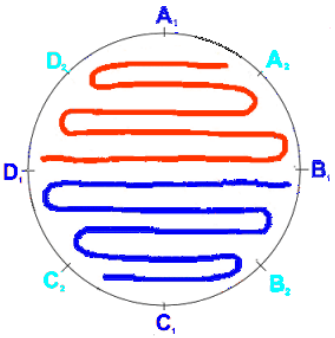
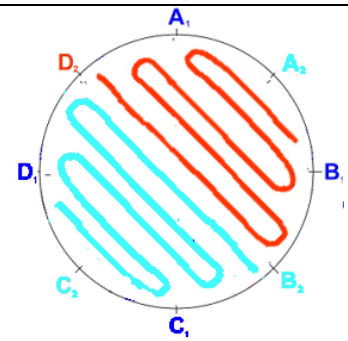
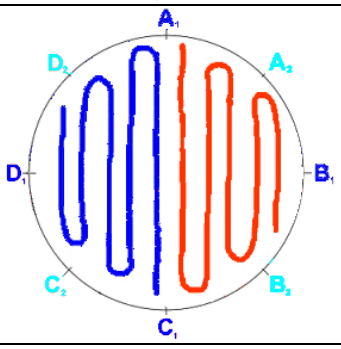
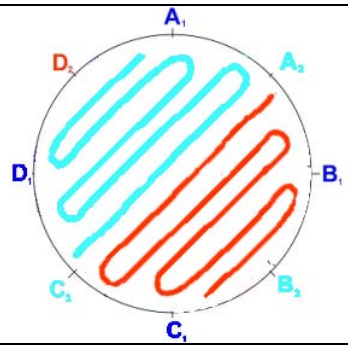
Total amount of water applied	a. Day 1	b. Day 2
20 litres (2 x 10 litres)		
40 litres (2 x 20 litres)		
60 litres (2 x 30 litres)		
80 litres (2 x 40 litres)		

Figure 4.3. Water application. a. Water application on day one. After each person has applied 10 litres, move 90 degrees clockwise and start again. B. Water application on day two. The initial starting point should be moved 45 degrees clockwise.

4.1 Potential sources of errors during the treatment of experimental plots

Potential errors during the treatment of experimental plots can occur:

- Identification and location of correct plot
- Amount of fresh water applied
- Spillage of fresh water during transport to the experimental plot
- Uneven application of fresh water across the plot

5 Location of sampling position within a plot

Sampling positions for each plot are generated by assigning a random compass bearing and distance (in cm) for each set of samples (i.e. 10 bearing/distance measures for each sampling occasion, Figure 5.1 and 5.2).

Figure 5.1. Sampling location within a plot

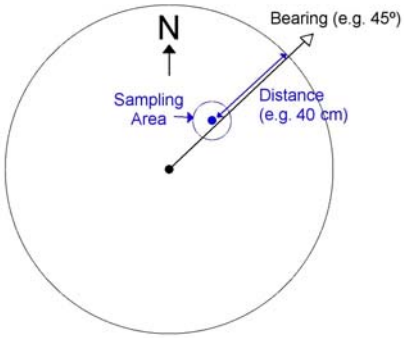


Figure 5.2. Schematic diagram of sampling location

6 Sample collection

All treatment samples should be collected 30 minutes after fresh water application. Control samples should be collected alongside, immediately before or immediately after the treatment samples.

Samples should be collected in the order and using the tube size described below (Figure 6.1, Table 6.1). To avoid possible cross-contamination between the sampling plots, the perspex tubes and plungers should be rinsed with filtered seawater after every use.

The perspex tube is pushed into the sediment to maximum sampling depth with one single action. Gentle rotation is acceptable but should be kept at a minimum (i.e. < 90°). If necessary, an unbreakable plastic lid or wooden board can be placed on the top of the tube which is then pushed into the sediment, using your knee for support. The lid or board should not form an air-tight seal on the tube; preferably, it should be perforated to relieve pressure.

1. Meiofauna: Samples should be collected using a 5.3 cm internal diameter perspex tube with 15 cm depth marked on the outside and the depth to which samples are taken should remain consistent. The rubber bung should not be in the tube when it is pushed into the sediment: Remove the bung from a tube, insert it to the desired sample depth and then carefully replace the bung. The perspex tube should not be excavated from the sediment, pull it out of the sediment carefully, supporting the bottom end. Great care should be taken when transferring sediment from the tube to the container – place the tube over a labelled container and then remove the bung to release the sediment. If any of the sample is lost, it should be rejected.

2. Chl a: Samples should be collected using a 3.6 cm internal diameter cut-off syringe to a depth of 2 cm. No slicing is required.

3. Salinity: Samples should be collected using a 3.6 cm internal diameter perspex corer to a depth of 15 cm and sliced in to vertical subdivision of fifteen 1 cm layers.

4. Particle size: Samples should be collected using a 3.6 cm internal diameter perspex corer to a depth of 15 cm. Sub-samples for organic carbon analysis should be taken from these cores by homogenising the sediment and collecting a 2 ml sub-sample.

5. Particle size: On four occasions (T_{-1}, T_0, T_1, T_{10}), an additional sediment core should be taken and sliced into 1-cm layers. Permeability will be determined from these four samples by calculations based on grain size data.

6. Sediment temperature: On each sampling occasion, sediment temperature should be recorded at three locations along the beach (adjacent to blocks). A thermometer should be inserted to 1 cm depth and temperature recorded on the log sheet after 1 minute.

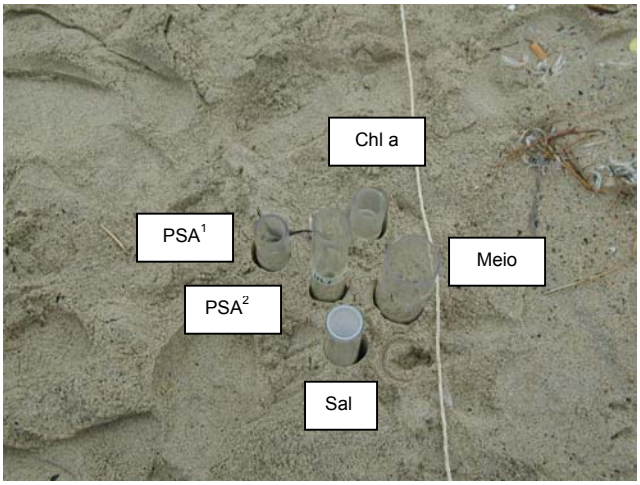


Figure 6.1. Sample collection

Table 6.1. Type of corer and sampling depth

Sample	Corer	Depth	Slicing
1. Meiofauna	5.3 cm ∅[3] Perspex tube	15 cm	No
2. Chlorophyll a	3.6 cm ∅[3] cut-off syringe	2 cm	No
3. Salinity	3.6 cm ∅[3] perspex tube	15 cm	15 x 1 cm slices
4. Particle size[1]	3.6 cm ∅[3] perspex tube	15 cm	No
5. Particle size[2]	3.6 cm ∅[3] perspex tube	15 cm	15 x 1 cm slices
6. Sediment temp	N/A	1 cm	N/A

[1] Collect a 2 g sub-sample of homogenised sediment for carbon analysis
[2] Only to be collected at T_{-1}, T_0, T_1, T_{10}
[3] Internal diameter

6.1 Potential sources of errors during sample collection

Potential errors during sample collection can occur:

- Choice of type of correct corer and sampling depth
- Use of rejection criteria
- Sample transfer from corer to container
- Labelling of sample, both internally and externally
- Method of preservation
- Sample storage

7 Timing of sample collection

The experiment should be carried out after springtime in March 2007. The frequency of rainfall and timing of sampling is shown in Figure 7.1 and Table 7.1. During the first week, rainfall is applied daily. A last rainfall treatment is conducted three weeks after T_0. The first seven disturbance events mimic a one-week episode of intensive rainfall. The last event is used to assess potential recovery from the cumulative effects of increased rainfall.

T_{-1} samples describe the initial situation and are collected 4 days prior to the set-up. T_0 samples are collected immediately before the first fresh water application. T_1 samples quantify an immediate physiological response; T_4 and T_7 samples quantify the effect of repeated rainfall after 320 l (T_4) and 560 l (T_7) of the fresh water have been applied to the treatment plots. T_9 and T_{10} samples measure the resilience of the communities. T_4 and T_8 samples are taken as a back-up and archived.

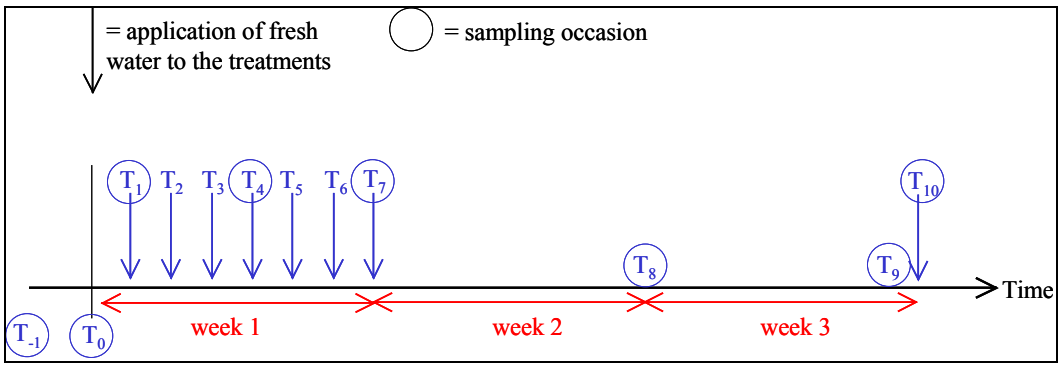


Figure 7.1. Timing of sample collection

Table 7.1. Timing of sample collection

Sample name[1]	Day of experiment	Total volume of fresh water [l] applied to date[2]	Time of sampling	Samples to be collected
T_{-1}	4 days before	0	4 days prior to beginning of experiment	Meio, Chl a, Sal, PSA (not sliced), PSA (sliced), Sed temp
T_0	Day 1	0	Immediately before 1st application	Meio, Chl a, Sal, PSA (not sliced), PSA (sliced), Sed temp
T_1	Day 1	80	30 mins after 1st application	Meio, Chl a, Sal, PSA (not sliced), PSA (sliced), Sed temp
T_2	Day 2	160	No sampling	N/A
T_3	Day 3	240	No sampling	N/A
T_4	Day 4	320	30 mins after 4th application	Meio, Chl a, Sal, PSA (not sliced), Sed temp
T_5	Day 5	400	No sampling	N/A
T_6	Day 6	480	No sampling	N/A
T_7	Day 7	560	30 mins after 7th application	Meio, Chl a, Sal, PSA (not sliced), Sed temp
T_8	Day 14	560	1 week after 7th application	Meio, Chl a, Sal, PSA (not sliced), Sed temp
T_9	Day 21	560	Immediately before 8th application	Meio, Chl a, Sal, PSA (not sliced), Sed temp
T_{10}	Day 21	640	30 mins after 8th application	Meio, Chl a, Sal, PSA (not sliced), PSA (sliced), Sed temp

[1] Coded by number of fresh water application
[2] Volume per treatment plot

8 Labelling of samples

All samples should be labelled with external labels, containing the following information: Sampling date, sample number (No.), beach, occasion, plot, sample type.

No. = unique code for each sample, consisting of the first letter of the beach followed by a consecutive number. For example, the first sample collected at Hel would be H0001, the second sample H0002 etc.

Meiofauna samples should also contain an internal label with the same information.

Date: 2007/04/17

No: H0001

Beach: Hel

Occasion: T1

Plot: C1

Type: Meio

Sample record spreadsheets have been prepared for each beach (see appendix). These should be used to print sample labels.

9 Sample preservation

When samples are taken in the field, it is often difficult or undesirable to carry large volumes of formalin to preserve the samples at the field site. However, if the samples have enough water to ensure they do not dry out, they can be left for up to 24 hours, depending on room temperature (< 20 °C) or up to 48 hours if refrigerated before adding formalin. Meiofauna samples should be preserved in 4 % buffered formalin (made from filtered (38 μm) formalin in filtered (38 μm) seawater or filtered (38 μm) fresh water). Buffer using Borax (sodium borate).

Particle size, total organic carbon and salinity samples should be transported from the field to the laboratory in their containers. There they should be immediately dried in an oven at 60° C pending transport to the University of Gent.

Chlorophyll a samples should be transported from the field to the laboratory in a cool box. They should be immediately transferred to - 20 °C storage pending transport to the University of Gent, where they will be transferred to - 80 °C.

10 Processing of samples and preparation of microscope slides

Processing meiofauna samples and making up slides should be carried out following procedures listed in the appendices. Where possible, each location should arrange for all extractions to be carried out by one scientist to ensure consistency.

At all stages of sample processing, regular cross-checks with a third party should be carried out to maintain quality.

10.1 Potential sources of errors during processing of meiofauna samples

The potential sources of errors during processing of meiofauna samples include:

- Spillage of sample when removing the lid of the sample container

- Excess pressure from water hose causing spraying of the sample
- Causing the sieve to overspill due to a blocked sieve by an overly silty sample
- Spillage of sample when transferring from sieve into decantation recipient.

It should be ensured that:

- The sieves used are undamaged
- The entire sample is washed from the sieve into the decantation vessel
- The extraction procedure is repeated the correct number of times

10.2 Potential sources of errors during the preparation of microscope slides

Potential sources of errors during slide-making can occur while:

- Taking of sub-samples (if applicable)
- Evaporation of the sample into glycerol
- Transfer of the sub-sample from the cavity block onto the slide (check under a compound microscope that no animals remain in the cavity block)
- Sealing of the slide (e.g. loss of sample material due to incomplete sealing)

11 Meiofauna identification

Ideally, identification of each meiofaunal taxa should be carried out by one scientist per location to ensure consistency of results. Regular consultation between colleagues engaged in meiofauna identification for the different experimental sites should be maintained.

Meiofauna should be identified to genus or species level using the available taxonomic sources. Any specimen retaining the head should be identified. If the head is not present, it should not be included in the data, even if the specimen can still be identified. If the identification of a specimen is not possible due to lack of information in taxonomic keys, it should be entered in the Manuela section on the NeMys database (http://nemys.ugent.be/manuela). Please ensure that identifyers are registered NeMys users beforehand. The following items should be archived on the NeMys database:

1) Slide reference of the unknown species.
2) Drawing of a type specimen in good condition and/or a digital photograph of the type specimen. A drawing should always be the minimum required. Photographs exclude a lot of information.
3) Name of the specimen (the lowest known taxon name with the addition of the identifyer and a number, e.g. *Sabatieria* sp. steyaert 1).

The type specimen and its drawing and/or digital photograph should be used for future reference within the Manuela community. An ‘unknown species’ form will be available on the NeMys database, detailing the type of information that should be recorded.

Both specimens that were alive and dead at the time of collection should be enumerated and identified. All specimens should be recorded on meiofauna recording sheets (see appendix). Specimens that had died prior to sample collection are generally characterised by (Figures 11.1 to 11.4):

- Decomposing or shrinkage appearance
- loss of internal features (e.g bulb, intestine)
- cleared with only cuticularised features (e.g. body ornamentation, amphids) visible

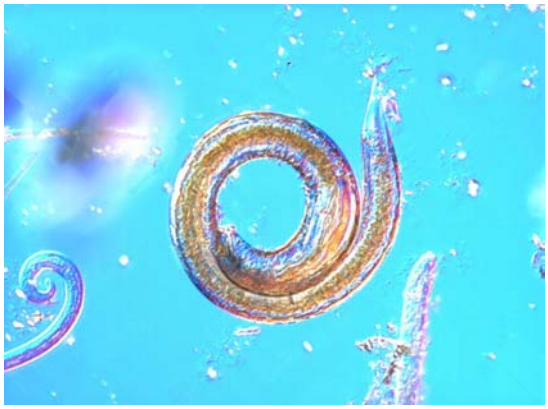


Figure 11.1. *Metachromadora remanei* alive prior to sample collection

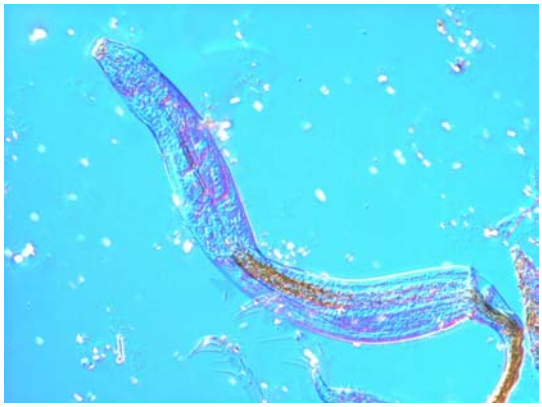


Figure 11.2. *Metachromadora remanei* dead prior to sample collection

Figure 11.3. *Terschellingia longicaudata* alive prior to sample collection

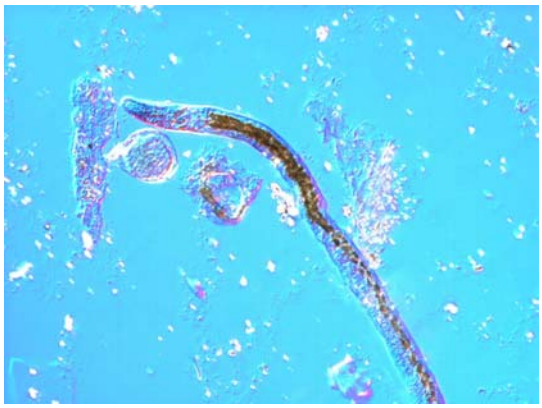


Figure 11.4. *Terschellingia longicaudata* dead prior to sample collection

All specimens should be recorded in three separate datasets: ‘alive’, ‘damaged’, ‘dead’.

11.1 Potential sources of error during meiofauna identification

It should be ensured that:

- All animals are counted
- The same animals are all given the same name

12 Retraceability of samples and results

When samples are being taken in the field, they should be labelled both internally, with a waterproof internal label, and externally on the container using a water and UV-proof pen. All samples taken should be recorded on the log sheet. All samples should be catalogued and stored in a suitable location.

All samples should be catalogued on an appropriate section in the Manuela database. Once both faunal and environmental samples have been analysed, the results should also be entered into this database, following a common procedure.

13 Appendix: Sampling and processing protocols

13.1 List of materials and equipment

This list of materials is made on the assumption that 3 groups of 2 people will carry out the raining treatment and the sampling of treatment and control plots and an additional person to recorder data.

General

- Camera
- Log Sheets
- Pencils and sharpener
- Permanent markers
- Spare Labels (external and internal)
- Spare sample containers (all sizes)
- Quality Assurance document (laminated)
- Tide tables
- Mobile phone
- Wet weather clothing
- First aid kit (including eye wash)
- Person/s trained in first aid
- Protective clothing (use of formalin)
- Temperature probe (air and sediment)

Positioning of plots

- Long ropes or metre tapes (beach dependent)
- 10 Sticks/gardening canes for marking the centre of the plots (buried to a suitable depth)
- Different coloured canes for treatments and controls
- Permanent labels for attachment to location ropes
- Level

Location of plots

- 2 – 3 Long ropes or metre tapes (beach dependent)
- Replacement sticks/garden canes
- Level

To trace plot outline

- 1.2m of rope attached to cane
- 1.2m rope used to measure distance between plots

Application of fresh water

- Fresh water (400 litres each day 80 litres per treatment plot)
- Wheel barrow / trailer to transport water / empty containers to and from the experimental site
- 3 x watering cans

Location of sampling position

- List of bearings and distances
- Compass
- 1.2m rope used to measure distance between plots
- Metre tape

Sampling of plots

- 3 (+ extra) cores tubes (diam. 3.6 cm): need to be marked at 15 cm
- 1 (+ extra) cut-off syringe (diam. 3.6)
- 1 core (+ extra) (diam 5.3 cm): need to be marked at 15 cm
- 1 x device to extrude sample
- 1 x device to slice sections (metal plate, spatula)
- 1 x device to subsample organic matter from PSA core (possibly cut off syringe)
- 3 (+ extra) stoppers for small cores
- 3 (+ extra) stoppers for big cores
- 85 1 litre (+ extra) recipients meiofauna
- 85 (+ extra) Petri dishes (plastic) chlorophyll a. Tape for taping replicates together
- 85 (+ extra) recipients particle size analysis (need to be of sufficient size)
- 85 (+ extra) recipients organic small vials (need to be of sufficient size)
- 85 x 15 plastic bags for salinity samples (need to be sealable)
- 3 buckets (for cleaning cores)
- Additional fresh water for cleaning cores tubes - or use filtered seawater from the beach
- External pre-printed adhesive labels for all samples
- Internal pre-printed labels for all meiofauna samples
- Wash bottles

Sample preservation

- 4 % formalin
- Containers for storing/safe transportation of samples
- Cool box and frozen ice packs

13.2 Sample record sheets, labels

Sample record spreadsheets have been prepared. These have been sent to the hosts of each experiment and should be used to print sample labels. An example is shown below for the beach at De Panne.

Sample Code	Beach	Occasion	Sample Type	Plot Type (Watered or Control)	Replicate	Date	Notes
P0001	De Panne	T^{-1}	Meiofauna	W	1		
P0002	De Panne	T^{-1}	Chlorophyll a	W	1		
P0003	De Panne	T^{-1}	Salinity	W	1		
P0004	De Panne	T^{-1}	Particle size analysis (not sliced)	W	1		
P0005	De Panne	T^{-1}	Particle size analysis (sliced)	W	1		
P0006	De Panne	T^{-1}	Meiofauna	C	1		
P0007	De Panne	T^{-1}	Chlorophyll a	C	1		
P0008	De Panne	T^{-1}	Salinity	C	1		
P0009	De Panne	T^{-1}	Particle size analysis (not sliced)	C	1		
P0010	De Panne	T^{-1}	Particle size analysis (sliced)	C	1		
P0011	De Panne	T^{-1}	Meiofauna	W	2		
P0012	De Panne	T^{-1}	Chlorophyll a	W	2		
P0013	De Panne	T^{-1}	Salinity	W	2		
P0014	De Panne	T^{-1}	Particle size analysis (not sliced)	W	2		
P0015	De Panne	T^{-1}	Particle size analysis (sliced)	W	2		
P0016	De Panne	T^{-1}	Meiofauna	C	2		
P0017	De Panne	T^{-1}	Chlorophyll a	C	2		
P0018	De Panne	T^{-1}	Salinity	C	2		
P0019	De Panne	T^{-1}	Particle size analysis (not sliced)	C	2		
P0020	De Panne	T^{-1}	Particle size analysis (sliced)	C	2		
P0021	De Panne	T^{-1}	Meiofauna	W	3		
P0022	De Panne	T^{-1}	Chlorophyll a	W	3		
P0023	De Panne	T^{-1}	Salinity	W	3		
P0024	De Panne	T^{-1}	Particle size analysis (not sliced)	W	3		
P0025	De Panne	T^{-1}	Particle size analysis (sliced)	W	3		
P0026	De Panne	T^{-1}	Meiofauna	C	3		
P0027	De Panne	T^{-1}	Chlorophyll a	C	3		
P0028	De Panne	T^{-1}	Salinity	C	3		
P0029	De Panne	T^{-1}	Particle size analysis (not sliced)	C	3		
P0030	De Panne	T^{-1}	Particle size analysis (sliced)	C	3		
P0031	De Panne	T^{-1}	Meiofauna	W	4		
P0032	De Panne	T^{-1}	Chlorophyll a	W	4		
P0033	De Panne	T^{-1}	Salinity	W	4		
P0034	De Panne	T^{-1}	Particle size analysis (not sliced)	W	4		
P0035	De Panne	T^{-1}	Particle size analysis (sliced)	W	4		
P0036	De Panne	T^{-1}	Meiofauna	C	4		
P0037	De Panne	T^{-1}	Chlorophyll a	C	4		
P0038	De Panne	T^{-1}	Salinity	C	4		
P0039	De Panne	T^{-1}	Particle size analysis (not sliced)	C	4		
P0040	De Panne	T^{-1}	Particle size analysis (sliced)	C	4		
P0041	De Panne	T^{-1}	Meiofauna	W	5		
P0042	De Panne	T^{-1}	Chlorophyll a	W	5		
P0043	De Panne	T^{-1}	Salinity	W	5		
P0044	De Panne	T^{-1}	Particle size analysis (not sliced)	W	5		
P0045	De Panne	T^{-1}	Particle size analysis (sliced)	W	5		
P0046	De Panne	T^{-1}	Meiofauna	C	5		
P0047	De Panne	T^{-1}	Chlorophyll a	C	5		
P0048	De Panne	T^{-1}	Salinity	C	5		
P0049	De Panne	T^{-1}	Particle size analysis (not sliced)	C	5		
P0050	De Panne	T^{-1}	Particle size analysis (sliced)	C	5		
:	:	:	:	:	:		
:	:	:	:	:	:		
:	:	:	:	:	:		
P0410	De Panne	T^{10}	Particle size analysis (sliced)	C	5		

13.3 Sampling log sheet

Log sheet

All plots located W C

All plots outlined W C

Application of 80L FW to treatment plots **(Time)**

Notes:

W1 W2 W3 W4 W5

Sample treatment plots **(Time)**
Bearing **(Degrees)**
Distance **(cm)**

Meiofauna (5.3cm diameter)
PSA (3.6 diameter)
Salinity (3.6 diameter)
Organics (sub-sample from PSA)
Chlorophyll a (3.6 cut-off syringe)
Permeability (3.6 diameter) T_{-1}, T_0, T_1, T_{end}

Treatment samples labelled (external and internal)

C1 C2 C3 C4 C5

Sample control plots **(Time)**
Bearing **(Degrees)**
Distance **(cm)**

Meiofauna (5.3cm diameter)
PSA (3.6 diameter)
Salinity (3.6 diameter)
Organics (sub-sample from PSA)
Chlorophyll a (3.6 cut-off syringe)
Permeability (3.6 diameter) T-1, T0, T1, Tend

Control samples labelled (external and internal)

Preserve faunal samples (4% formalin) ☐

Chl a samples placed in cool box ☐

T_{end} sample none-sampled control ☐

Date_____ Beach_____ Sampling occasion_____

Start time_____ Finish time_____

Sampling groups

Plot locating team Rain team Sampling team

Weather observations

Weather description

Wind direction_____ Air temp_____ Sediment temp_____

_____

_____

_____

_____

Site observations

Experimental site description before any action

Litter (count /description)

Other observations

Lost samples

13.4 Beach sampling protocol

This procedure should be used by all laboratories for the MANUELA experiment to ensure data compatability. It is a detailed description of the procedure developed by all the MANUELA representatives at the Sines Workshop.

Determining the position of the plots

1. Use a rope or tape measure that does not stretch (many ropes will stretch when pulled or when wet).
   Suggested types of rope: Boat rope (what grade?), nylon, measuring tape.
2. Mark the tying position (starting point) on the rope by sewing in to it a piece of coloured thread or wire.
3. Then attach masking tape or other waterproof material to the rope and write on it the location and height of rope attachment with a waterproof pen.
4. Preferably lie the tape gently along the ground, so that it lies straight but is not slack and not pulled tightly.
5. The same people should make the measurements on each occasion – each person may hold and pull the rope differently.
6. Having determined the position of the plot, mark its position on each rope by threading through coloured string or thread such as wire. Then attach masking tape to the tape/rope and write on it with a waterproof pen the plot designation.
7. Insert a 1 metre long wooden cane into the centre point of the plot. The cane should not protrude more than 5 cm from the sediment surface.
8. Repeat for each plot, recording their position on a map.

Finding and marking the plot for the experiment

9. Make a note of the time on your log sheet.
10. Use the rope measurements to confirm the position of the plots. These should help to locate the middle posts in each plot and confirm their designation.
11. Take care to repeat the same method used when determining the position of the plots. Preferably lay the tape gently along the ground, so that it lies straight but is not slack and not pulled tightly.
12. To mark the plot, tie a loop in one end of a rope and put this over the centre pole. Measure 120 cm from the centre and use the rope as a guide to mark a circle in the sand using your finger.
    - Do not pull the rope to tightly – it may dislodge, altering the size of the plot and possibly disturbing the sediment.
13. Remove the rope.
14. Whilst marking the plot, limit the amount of disturbance you make by treading on the sampling area. It is acceptable to walk briefly on the sampling area because this should hopefully equate to normal disturbance.

Watering the plots

It may be necessary for the taller members of the team to do this part – they can more easily reach the centre of the plot and (in theory) should be more able to reach out with the heavy watering can.

15. Divide the plot into quarters, marking the divisions outside the plot area.
16. Place 20 litres of water at each of these points.
17. To begin, the two waterers stand opposite each other across the plot with one watering can each. The cans should be large enough to leave space when they are filled with 10 litres of water
18. Fill each watering can with 10 litres of water. Do not be tempted to pour the water in two 5 litre applications; this may lead to an uneven distribution of water over the plot.
19. Make sure the watering spout on the can is secure – it can fall off!
20. Make a note of the time on the log sheet.

21. Standing at the edge of the plot, each waterer should together gently pick up their watering can, taking care not to spill any water.
22. Each person then gently pours the water over the plot, starting at the edge of the plot, working inwards (so that you are not stretching to the very middle with a full, heavy can).
23. Pour so that the can swings from one side of the plot to the other, so that the whole plot area receives an even application of water.
24. Make sure that you pour within the half-circle marked in front of you, though some over lap across the boundary line will prevent a 'dry spot' in the middle.
25. The two waterers should try to pour at a similar rate and at a similar height from the sediment surface.
26. Do not pour too quickly – this may cause pooling on the sediment surface. On a sloped surface it may also cause run-off, which would result in an uneven application of water over the plot.
27. Now move 90 degrees clockwise.
28. Repeat steps 17 – 27 until all the water has been poured on the plots. On each occasion take care to stand at opposite marked quarter points and to water up to and between the vacant quarter points.
29. Each person will have poured 10 litres of water from each marked quarter pointed.
30. Moving around the plot is very important – it limits the effect of different application methods between the two waterers. It also dissipates any effect of water drift cause by wind.

Taking the cores

31. Ensure that you have all the storage pots required for the sampling before you start. They should be labelled externally, and preferably should already also contain internal labels (only meiofauna with an internal label).
32. Check that the core tubes are marked with the required depths and that an arrow indicates the direction of insertion.
33. Determine the sampling point by using the compass bearing and distance pre-calculated randomly in excel. (Samples should be taken between 10 and 100 cm from the centre point.)
34. Take the bearing from the centre point of the plot, using the rope to mark it.
35. Measure the distance from the edge of the plot along the bearing (rope) toward the centre point.
36. Note the time coring begins.
37. First, insert the meiofaunal core tube into the sediment nearest to the located point. Insert a stopper into the end of the tube and leave it in the sediment.
38. Then insert each of the remaining cores required, closing them with stoppers and leaving them in place before starting the next.
39. The tubes should be inserted in one motion:
    - Standing with your body over the tube and your knees bent, slowly and gently push the tube into the sediment.
    - If you encounter resistance, gently twist the tube in one steady motion (no more than
    - about 180 degrees).
    - It is very important that the tubes are inserted vertical to gravity.
    - Do not cover the end of the tube as you push into the sediment, the air trapped in the tube will push onto the sediment and may cause it to be compacted.
    - Do not excessively twist the tube, this will disturb the sediment.
    - Do not rock the tube from side to side – this disturbs the sediment and may compact it as the tube enters the sand.
    - Do not hammer the tube into the sediment, this may also compact the sediment.
40. Before removing the cores make sure you have the correct sample containers ready. Have one person preparing and holding the pots and one person removing the cores, and slicing (if required).

41. Do not remove a core from the sediment until its receiving pot is open – minimise the time between removing the sample and putting it into an appropriate, pre-labelled container.
42. Always have the extruder ready – you never know, it may be required even if you have beautiful cores!
43. Remove, slice (if required) and store each core separately and in the following order:
    a. Meiofauna
    b. Chlorophyll a
    c. Salinity
    d. Sediment analysis
    e. Additional particle size analysis (if required)
44. Note the time that each core is removed.
45. Remove a core gently and vertically, limiting disturbance to the surrounding sediment. The stopper should hold the sediment in the core, but move it gently and transfer it to a sample pot as quickly as possible.
    - Always keep the core tube vertical.
    - Do not shake or invert the tube; this may lead to loss of water from the sample (which will take animals with it!)
    - Shaking or inverting the tube may also disturb the sediment, this is particularly important if the sample needs to be slices into depth horizons (for example, the salinity sample), or if the core is too long and needs reducing.
46. Always remember to measure the depth of the core and only collect the required depth.
47. To reduce the depth of a core that is too long either;
    a. Place the tube on an extruder, so that the bottom of the sediment rest on it and the top of the sediment is uppermost.
    b. Remove the stopper from the core tube.
    c. Put a second core tube directly over the core tube containing the sediment sample and gently push out the required amount of sediment into it. Do this by holding the sample core tube and gently pushing it over the extruder. Do not twist the sample over the extruder.
    d. Now slice the core using a flat metal plate.
    e. Wash the sample into the sample pot and rinse the inside of the core tube and the side of the metal plate which came into contact with the sediment retained.
    f. Discard any additional material and wash the two corers, metal plate and extruder.

    OR;
    a. Gently lift the stopper from the core tube allowing the excess sediment to fall from the bottom of the tube. It is very important to do this slowly so you can 'slide' the column of sediment out smoothly until it reaches the required core length level with the bottom of the core.
    b. Make sure you do this away from the plot area!
    c. This method sounds to be very simple, but extreme care should be taken to stop the whole core slipping out of the tube!
48. Take all the samples to the laboratory as quickly as possible. Samples are stored in the following manner:

Sample	Preservation method	Storage Pot
Meiofauna	4 % foramlin (fill pot to neck)	1 litre pot
Chlorophyll a	Freeze at – 20 ºC	Sufficient size
Salinity	Dry at 60 ºC	Sufficient size
Particle size	Dry at 60 ºC	Sufficient size
Organic carbon	Dry at 60 ºC	Sufficient size

49. On each of the following sampling occasion, the starting position for watering should move 45 degrees clockwise.

13.5 Random distances and bearings tables

Tables are provided giving a series of randomly generated distances and bearings for the location of sampling areas within plots. To simplify this process and provide a grid of locations which relates to the areas disrupted during sampling, distances have been set at a resolution of 20 cm (20, 40, 60, 80 and 100 cm) measured inwards from the edge of the plot and bearings at a resolution of 15° (0, 15, 30.. ..345°) from magnetic North. It is recommended that the tables are not accessed in the same way and that sampling teams choose randomly which columns or rows to use first.

Manuela Beach Experiment:									
Randomly Generated Sampling Location Distances (cm)									
80	100	40	80	40	40	80	20	80	40
100	100	100	60	40	80	100	40	60	100
100	60	60	100	20	20	60	20	20	60
80	40	100	100	40	100	80	20	60	20
100	80	100	20	20	60	20	100	40	80
80	20	100	100	80	80	60	80	40	80
40	40	80	80	80	100	60	60	100	60
40	20	40	80	60	60	80	60	100	20
40	20	60	20	80	20	80	20	20	60
40	60	60	80	20	80	40	80	40	20
100	40	60	80	80	20	60	100	80	80
40	60	40	60	100	80	100	20	20	80
60	100	40	40	80	20	40	60	100	40
80	100	80	80	20	20	40	40	40	100
20	20	60	60	40	60	40	60	40	40
100	40	100	40	80	20	100	80	100	20
100	100	100	100	20	60	40	20	60	60
80	60	100	80	60	80	60	60	20	80
100	60	40	20	100	100	80	80	100	80
80	60	80	80	80	60	80	40	60	40
20	80	40	20	100	40	20	100	20	40
60	80	100	60	100	40	40	100	60	20
80	40	40	100	100	40	80	100	20	80
80	100	60	100	20	80	20	20	40	40
40	40	40	20	40	60	60	60	40	20
60	100	60	20	40	40	100	100	100	60
40	60	80	60	20	20	40	20	60	40
80	20	20	80	100	60	60	20	60	80
20	40	40	100	20	40	40	60	100	60
80	60	60	20	40	20	80	20	20	40
20	20	20	40	20	60	80	20	80	40
40	40	20	80	80	100	100	80	20	20
60	20	60	40	80	60	100	80	100	60
40	20	100	60	60	80	100	100	100	40
100	40	20	20	80	60	80	60	20	80
60	20	100	60	40	100	40	20	40	100
40	100	80	40	40	20	80	80	40	100
80	80	40	80	80	80	60	80	80	80
60	100	60	60	80	60	40	100	80	20
60	40	20	20	100	80	100	80	20	20
40	60	60	20	40	20	80	40	100	100
40	100	80	60	100	20	60	100	100	80
40	20	60	60	20	20	40	40	20	20
100	100	60	20	100	40	40	20	60	20
80	20	20	40	80	60	80	60	60	100
100	60	100	100	60	80	20	60	100	100
80	20	20	60	40	40	60	80	20	80
100	20	100	60	100	60	40	20	60	100
60	40	60	40	60	20	80	40	20	100
40	100	100	100	100	40	60	80	80	100

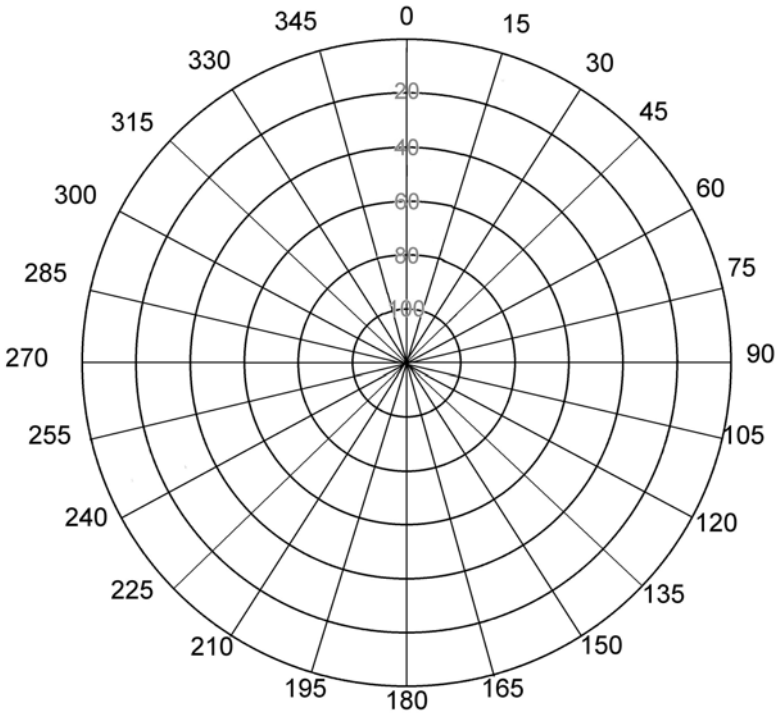
Manuela Beach Experiment:									
Randomly Generated Sampling Location Bearings (°)									
135	255	180	45	105	180	345	315	240	120
285	330	330	240	90	120	315	135	150	75
240	15	240	60	150	270	135	30	345	90
135	180	75	0	90	90	135	315	345	30
120	285	105	210	120	120	270	90	90	0
330	45	120	105	195	270	15	30	330	300
270	75	255	15	135	60	285	165	30	60
15	255	165	165	135	120	75	105	180	180
75	0	0	90	300	135	255	75	30	120
330	135	195	105	75	345	315	30	240	210
60	300	345	345	60	45	30	195	300	315
240	300	270	285	180	195	195	15	45	135
180	45	255	255	255	210	0	60	90	210
15	270	210	330	300	105	240	270	60	270
225	150	345	75	225	0	225	75	105	345
210	255	180	285	330	180	105	150	300	45
30	165	210	0	30	105	240	225	315	240
270	285	300	330	345	240	315	60	330	240
270	180	330	45	60	225	330	225	300	75
255	45	330	270	240	315	30	60	150	90
165	225	270	345	195	30	120	300	240	45
225	315	165	60	210	75	105	270	60	60
270	105	210	120	15	45	300	30	75	120
75	75	150	255	210	180	210	75	150	255
285	225	345	195	300	135	45	345	255	15
270	255	0	120	15	195	210	285	180	195
150	225	0	75	90	105	105	315	30	165
195	180	0	105	315	60	240	0	225	0
30	90	345	135	165	150	165	45	255	240
120	135	315	315	225	285	330	165	90	330
225	300	0	225	150	270	0	90	225	300
345	345	315	150	285	210	165	105	135	90
90	105	285	135	240	120	180	60	195	240
0	135	285	165	255	300	150	90	180	330
45	210	300	330	285	45	300	180	225	15
45	255	15	270	210	45	15	270	195	210
120	300	165	135	315	225	15	195	195	15
255	285	150	120	240	45	75	165	270	0
165	210	90	60	120	105	15	30	195	150
30	345	285	195	15	120	225	165	180	75
180	105	315	30	180	210	150	150	120	30
0	240	195	90	90	0	135	270	255	330
225	315	15	150	0	285	105	15	285	45
135	180	135	60	285	195	330	255	60	60
75	45	30	315	30	330	120	210	90	315
150	285	255	150	165	345	195	135	300	210
165	30	330	345	180	60	15	165	45	240
255	210	315	15	0	75	300	330	285	195
300	105	45	15	150	120	75	225	225	150
90	165	60	0	270	105	345	240	345	165

13.6 Plot sampling history

The following sheet is intended to provide a simple graphic means of assessing the sampling history of an individual plot. The distance and bearing used to identify each sampling occasion can be recorded in the field or transferred from the Sampling Log Sheet (section 13.2). The sampling location can then be marked (T -1, T 0, T 1 etc) on the "web" diagram of the plot. This will provide a simple, graphic means for the sampling teams to assess if sampling has already taken place at a location. In addition, if areas are known to have been heavily disturbed or disrupted, it will also be possible to mark such areas on the diagram for future reference. One sheet should be used for each plot and there are spaces for recording the beach, plot number and treatment. Each data entry should be initialled by the recorder making the entry.

Manuela Joint Action Field Experiment:	
Plot Sampling History	
Beach	
Plot	
Treatment (Control/Water)	

Sampling	Distance (cm)	Bearing (°)	Recorder	Sampling	Distance (cm)	Bearing (°)	Recorder
T -1				T 7			
T 0				T 8			
T 1				T 9			
T 4				T 10			



13.7 What if?

Should any of these incidents occur make a note on your log-sheet!

Plots

- **The landmarks are destroyed?**

You still might have

- visible plot marker
- different visible plot markers for rain and control plots

- **The plot site is not found?**

Has to be avoided: Triple security by

- visible plot marker
- rope technique (two to three ropes)
- different visible plot markers for rain and control plots
- Sines: Metal piece dug into sediment (can be detected with metal detector)

- **A plot/the whole site is flooded?**
  - go on with raining/sampling scheme
  - make notes on your observations

- **A plot is destroyed?**
  - carry on, make a note

Cores

- **The sand is too dry and falls out of the core?**
  - immediately close the core from underneath, stopper, hand

- **The cutting of slices doesn't work and the sample is lost?**
  - if you lose a slice, then omit the slice and carry on slicing the rest of the
  - core
  - if you lose a whole core sampling should only be repeated within the same plot when otherwise less than four replicates per treatment would be available

- **A device is destroyed?**
  - always take enough extra devices with you!

Water

- **We spill water?**
  - take spare water with you! If the spillage happens during irrigation do not try to estimate the volume of water lost (unless it is a whole watering can or container), continue adding the remaining water and make a note (estimate the volume lost). Spill outside or inside the plot?

- **The spout falls off?**
  - make a note and put the spout back, this time fix it properly (tape the spout to the can, but make sure both are dry first)

- **Part of the crew is ill or not available?**
  - have a list of spare people /substitutes
  - prioritise treating all the plots, rather than sampling, if you are running out of time

Samples/sampling

- **A sample is lost?**
  - make a note (see above)

- **You miss the sample time and it is impossible to wait for the next high tide? Or if you loose a lot of time finding the plots?**
  - treatment has priority, rather rain than sample!

- **The plots get covered with holes from sampling which drain the irrigated water?**
  - holes might slump during following irrigations, might be not so much of a problem (natural rainfall will do the rest)

- **You confuse the cores and use a core of wrong diameter?**
  - make a note on your log sheet, report it to analysing team
  - if you spill /confuse/ miss a sample send in an empty plastic bag /recipient with the correct label to the analysing team

Else

- **Part of the crew is ill or not available?**
  - always have a list with substitutes you can call in case you lack people for the field

13.8 Decanting

This procedure should be used by all laboratories for the MANUELA experiment to ensure data compatability.

It is adapted from the methods used by Dr's Barnes and Ferrero at the Natural History Museum, London.

Everyone has their preferred way of setting up the sink, so you will need to try out where to put equipment. Practice decanting with just water before working on your samples. This is particularly important because the glass cylinder is heavy and can easily break.

Formalin removal

1. Don a laboratory coat.
2. Label a small sample pot and lid with the sample details in permanent marker pen. (Sample code, your initials, and the date as dd/mm/yy).
3. Rinse equipment with filtered tap-water – 2-litre cylinder and stopper; 10 and 20 cm diameter 38 µm mesh sieves; spatula; large spoon; wide-necked funnel.
4. Put the 2 litre cylinder in the corner of the sink and put the wide-necked funnel in it.
5. Check that wash bottles with distilled and filtered water are full.
   - Use the 10 cm diameter sieve to refill the filtered tap-water bottles. Keep this sieve for filtering tap water.
6. Don gloves, safety glasses and a formalin respirator.
7. Work at a ventilated sink.
8. Wash the outside of the sample pot with tap-water to remove any dust.
9. Prepare your waste formalin container. Remove the screw-cap lid and insert a funnel. Put the container into the bottom of the ventilated sink.
10. Put the 20 cm diameter 38 µm mesh sieve into the funnel of the waste formalin container.
11. Carefully remove the lid from the sample pot, ensuring there is no spillage. Rinse the inside of the lid and put to one side.
12. Through the 20 cm diameter 38 µm mesh sieve, slowly pour-off the formalin from the sample into the waste formalin container. Pour slowly to reduce disturbance to the sediment (it can clog the sieve) and to allow drainage of the funnel. If you pour too fast you may increase your risk of exposure to formalin.
13. Remove the sieve and funnel lid and close the waste formalin container.
14. Any material retained by the sieve should be washed with filtered tap-water and rinsed into the 2 litre cylinder.
15. Wash the sample with filtered tap-water to remove excess formalin:
16. If you use internal paper labels remove this next using forceps. Rinse any sediment on the label and forceps back into the sample pot using filtered water. Put the label with the lid to one side.
17. Add filtered tap-water to the sample until it is about one third full and not beyond the half-way mark to reduce the risk of splashing.
18. Gently agitate the sample with a spoon, so that the sediment becomes suspended in the water. If necessary, gently break-up larger clay aggregations. Any material stuck to the spoon must be washed back into the sample container with more filtered tap-water.
19. Put the funnel back into the waste formalin container and replace the 20 cm diameter 38 µm mesh sieve.
20. Allow the sample to rest until most of the sediment has settled out.
21. Then through the 20 cm diameter 38 µm mesh sieve, pour-off from the sample as much liquid as possible into the waste formalin container. Again pour slowly to reduce disturbance to the sediment (it can clog the sieve) and to allow drainage of the funnel.
22. Remove the sieve and funnel and close the waste formalin container.
23. All material retained on the sieve should be washed with filtered tap-water and rinsed into the 2 litre cylinder.

24. Most of the formalin should now have been washed out the sample. Cover the sample with filtered tap-water and close the lid. Put the sample to one side at the back of the ventilated bench.
25. Now thoroughly wash the funnel and the outside of the formalin container with plenty of tap water and put then both out of the way (under the bench).

Preparating to decant

Everyone has their preferred way of setting up the sink, so you will need to try out where to put equipment. Practice decanting with just water before working on your samples. This is particularly important because the glass cylinder is heavy and can easily brake.

26. Rinse the sample lid into the 2-litre cyclinder.
27. After washing formalin from the sample (steps 1 – 24) wash the sample from the container into the 2 litre cylinder using filtered tap-water.
28. If the funnel becomes blocked use a long spatula or forceps to clear it. These should be rinsed with filtered tap-water over the cylinder.
29. Large stones should be removed with forceps and the stones and forceps rinsed with filtered tap-water over the cylinder. Put the stones put back into the original sample pot.
30. Now thoroughly rinse any material on the (inside and out) funnel into the glass cylinder with filtered tap-water. Put the funnel to one side.

Sample decanting

31. Top-up the measuring cylinder with filtered tap-water to 2 litres, rinsing the cylinder neck to remove all sediment particles.
32. Securely fit the correct plastic stopper.
33. The sample should now have only trace amounts or 'no' formalin in it. Remove your gloves to ensure you have a good grip on the glass cylinder. You may also remove your goggles and mask if you wish.
34. First check that you have enough space around you, then take hold of the stopper and neck and the base of the cylinder. Stand back from the sink and invert the cylinder five times. Make sure that all the sediment is suspended in the water column, particularly on the first invertion.
35. Put the cylinder down in the bottom of the sink. Start timing immediately, the sample should settle for 30 seconds <u>only</u>.
36. During this 30 seconds, carefully remove the stopper and rinse any material on it in back into the cylinder with filtered tap-water. Also, rinse the uncovered sides on the inside of the cylinder to ensure that all material is suspended in the water column.
37. At 30 seconds, carefully pick up the cylinder and resting it in the crook of your arm with you hand near the neck of the cylinder, slowly and gently pour the water through the 38 μm mesh sieve.
    - Holding the cylinder with you hand near the neck will give you more control over the speed of pour-off.
    - Resting the cylinder on your hip for support can also be helpful.
    - Holding the sieve at a slight angle away from you can reduce the chance of 'splash-back' and aid sieve drainage.
38. Maintain a steady flow of water so that the sediment that has settled out of the water column is not disturbed.
39. When nearly all the water has been poured-off, and as the settled sediment moves towards the neck of the cylinder, stop pouring, twisting the cylinder as you do so in order to prevent spillage.
    - <u>If the sieve becomes blocked stop pouring</u>.
      Gently tapping the side of the sieve or gently 'strumming' or stroking the bottom of the sieve will encourage water flow. With filtered tap-water rinse the material retained on the sieve into the red-lidded sample pot.

Complete the pour off to 'clean' the water column and again rinse material remaining on the sieve into the red-lidded sample pot with filtered tap-water.

- If the sieve becomes blocked so that (approximately) the full 2 litres cannot be poured from the cylinder, the decant should not be counted.

40. After a complete decant (i.e. when an entire column of water is poured off without stopping), either;
    a. If the sieve is full or blocked, use filtered tap-water to clean the material retained on the sieve and rinse it into the small sample pot.
    b. If only a small amount of material is retained on the sieve, wash the material to one side of the sieve and continue with the next pour-off. In this case it is very important to keep checking the sieve to make sure the material does not dry. This may not be possible if the ambient air temperature is very hot.
41. Ten full decants should be completed for each sample.

Archiving the sample and residue

42. YOU SHOULD WEAR GLOVES AND GOGGLES AND A FORMALIN RESPIRATOR WHEN USING FORMALIN.
43. All the material retained for picking after the 10 decants should be washed back on to the 38 μm mesh sieve and rinsed thoroughly with distilled water. It should then be washed back into the small sample pot with 4 % formalin, and covered by at least 75 ml of formalin.
    - It is very important to thoroughly check the sieve to prevent loss of fauna.
44. Insert paper and secure the lid.
45. Dry the outside of the small sample pot and add an external (adhesive) label, note in pencil initials, and the date you did the decant. Put the sample to one side for either ludoxing or picking, as appropriate.

Archive the sediment remaining in the cylinder so it can be checked later if necessary:

46. Use distilled water to wash the material from the cylinder back into the original large pot: Use as little water as possible to rinse all the material to the bottom of the cylinder and then gently pour it into the large sample pot, trying to keep the sediment to one side of the cylinder.
47. Keeping the cylinder inverted in the sample pot, use a full, inverted filtered-water wash bottle to wash the remaining sample into the sample pot.
48. At this point put on gloves, safety glasses and formalin respirator.
49. Pour-off as much water as possible from the sample container through the 38 μm mesh sieve. Any material retained on the sieve should be rinsed back into the pot with 4 % formalin.
50. Put the internal paper label back into the pot and fill with 4% formalin. Secure the lid.
51. Use a permanent BLACK marker pen to write on the side and lid of the sample pot 'Extracted', your initials and the date (dd/mm/yy).
52. The sample residue should be transferred to storage.

Before leaving the lab

53. Ensure that all equipment and sides are thoroughly cleaned and rinsed with distilled water. This is to ensure that no cross-contamination occurs between samples.
54. Deal with waste formalin as appropriate for your laboratory.
55. The last job is to decide what to do with the sample extract in the small sample pot. If it is more than ABOUT 2 mm deep, centrifugation with ludox will be necessary. Whilst centrifugation may be time-consuming, picking a sample that is deep with sediment/detritus is more so. To some extent this will be dependant on the sediment from you sampling area – you will need to experiment.

13.9 Making a picking pin

This procedure is used by Dr's Barnes and Ferrero at the Natural History Museum, London and is included as a suggested method

Pins can be made using an entomological pin or eyelash or eyebrow hair. You will need a range of sizes (length and width) and shapes.

1. Don a laboratory coat.
2. Melt wax in a pyrex dish at about 65 ºC (this depends on the melting point of your wax, we recommend a wax with a high melting point).
    - Melting the wax too quickly, or having the wax at too high a temperature for any period of time will denature it! This makes the wax brittle.
3. ONCE THE HOT PLATE IS SWITCHED ON DO NOT LEAVE IT UNATTENDED.
4. Have an entomological pin and glass pasteur pipette ready.
5. Prepare storage containers for all taxa to be potentially picked before starting a sample.
6. Label all pots and slides with an external adhesive labels.
7. Petri-dishes and cavity blocks should be labelled with a permanent marker on the lid, cavity blocks should also be labelled on the side.
8. Allow the wax to cool before using the pin – put it under cold running water if necessary. You may want to use forceps to pinch-over the point of the entomological pin to ease picking.
9. Ensure that the wax heater is turned off after use and is not left unattended whilst switched on.
10. If you must leave the room whilst the heater is still hot – please make sure there is another member of staff to look after it.

* Some people like to use super-glue rather than wax to fix the pin in place, but once the pipette is broken, the entomological pin is lost.

* Also, metal holders in which the pin is clamped in place are available, but these can unscrew during use.

13.10 Picking

This procedure is used by Dr's Barnes and Ferrero at the Natural History Museum, London and is included as a suggested method

Personal protective equipment
- Laboratory coat

Equipment Required
- Mounted entomological pins
- 1 x Cavity block (Nematodes)
  Pots for all other taxa

Chemicals Required
- Dehydrating solution (nematodes)
- 4 % formalin (tardigrades)
- 70 % alcohol
  (copepods and other taxa)

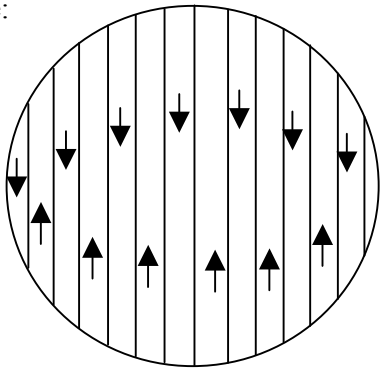
Set-up

1. To facilitate enumeration and picking, samples are divided into 8 parts and split into Petri dishes.
   We usually have Petri dishes containing 1/8th, 2/8th and 3/8th of a sample so that the 'best' dish can be picked (A dish with an abundant number of animals, but not too much detritus – Too many animals or too much detritus increases the difficulty of enumeration). If necessary scan a sample before picking.
2. Examine samples under a binocular microscope using x 10 eye-pieces and a 3.2x objective (or equivalent).
   Use higher magnification as necessary to confirm identification of the smallest fauna.
3. Pick animals using either an entomological pin or eyelash/eyebrow hair mounted in a glass Pasteur pipette.
4. Lines are scored on the underside of the Petri-dishes to facilitate picking. Lines should be not more than about 8 mm apart – you should be able to see a line on each side of your field of view.
5. Prepare storage containers for all taxa to be potentially picked before starting a sample.
6. Label all pots and slides with an external adhesive labels.
7. Petri-dishes and cavity blocks should be labelled with a permanent marker on the lid, cavity blocks should also be labelled on the side.
8. If you are working on your animals immediately, pick copepods into Lactic Acid on a cavity slide. Lactic acid absorbs water on contact with air and therefore may expand over time. Use only a small drop of lactic acid placing it directly in the centre of the cavity on a slide. Each slide should be stored in a separate petri-dish to protect it.
9. If you are storing specimens for a later date, put copepods in alcohol in a 2 ml pot.
10. Nematodes should be picked into a watchglass (cavity block) containing dehydrating solution (90% distilled water, 5% alcohol, 5% anhydrous glycerol and phenol crystals).
11. All other taxa should be picked into 2 ml pots containing 70 % alcohol or 4 % formalin – we have had problems picking some taxa into alcohol, particularly Tardigrada.

Picking the sample

12. Use a pin to evenly distribute the sample in the Petri dish.
13. Use a pin to add a very small amount of detergent (washing-up liquid) to the Petri dish. This reduces the surface tension and makes it easier to remove animals.
14. Starting at one side of the petri dish, follow the rows scored on the under-side of the dish and enumerate all animals encountered, picking animals as required.
15. When moving through the dish, be sure to use your pin to move aside all detritus and sediment. It can be surprising how a relatively large animal can hide under a small sand grain and how moving an animal relative to the incident light can make it more visible.
16. Pick animals using either an entomological pin or eyelash/eyebrow hair mounted in a glass pasteur pipette.

Picking through a sample:



17. You will find it useful to have more than one pin size. Using small, softer pins for scanning will reduce the number of scratches on the bottom of a dish, whilst bigger pins will be useful for picking larger animals like foraminifera.
18. Whilst it may be tempting to use a pipette to collect some of the more difficult fauna, try to avoid this. The water collected with the animal will reduce the concentration of formalin/alcohol the animals are preserved in and may lead to their deterioration.
19. All samples and taxa storage (slides / pots / cavity blocks) should be covered when not being used/picked into (ie. Over lunch/break times, overnight).
20. When you have completed picking the nematodes transfer the cavity block to the desiccator.
    - The cover should be left to one side to allow evaporation of water from the dehydrating solution.
    - Leave the sample in the desiccator for at least 2 days to allow complete water evaporation before slides are made.
    - Do not leave samples in the desiccator for more than 2 weeks. This increases the risk of fungal growth.

*It is important to regularly check the desiccating crystals and dehydrate them

21. Once picking is complete, use alcohol-proof internal labels in taxa pots.
22. It is usual to pick / count 3 sectors from the sample splitter to allow a good estimate of total sample abundance.

After Picking

23. After the sample has been enumerated/picked, any material remaining in the petri-dishes should be archived. For each sample, wash material from the Petri dishes onto a clean 45 µm mesh sieve with distilled water. Rinse all material back into its pot with fresh 4% BUFFERED formalin and store for reference. You might want to look for extra specimens later, some one else might want extra taxa, or you might be concerned about your data and need to double check the abundance results.
24. The Petri dishes may become scratched through use. After you have fully archived your samples check the quality of the Petri dish.
    - If it is ok, clean it with detergent and then rinse with distilled water and leave to dry or,
    - if you consider it to be excessively scratched dispose of it so that it is not accidentally used by another person.

13.11 Making slides

This procedure is used by Dr's Barnes and Ferrero at the Natural History Museum, London and is included as a suggested method

Personal protective equipment
- Laboratory coat

Chemicals Required
- Anhydrous glycerol
- < 50 ml Paraffin wax in Pyrex dish
- 80 % IMS

Equipment Required
- Mounted entomological pin
- Hot plate
- 1-1.2 mm thick slides*
- no. 1 (19 mm diameter) coverslips**
- Wax-ring applicator (Mounted copper tubing)
- Slide forceps
- Tissues (white and blue)
- ~63 µm glass beads
- Glass dropper

* Slides and cover-slips should be stored in IMS, or wiped with IMS before use

** This size is for 10 – 20 specimens per slide

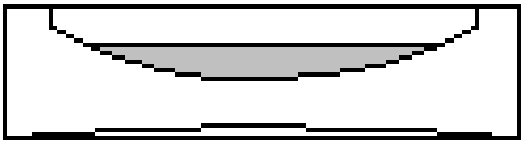
Preparation

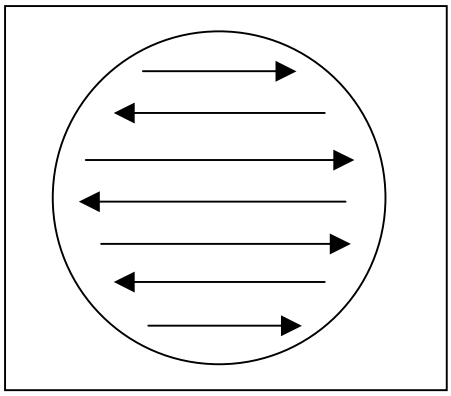
1. If possible switch the hotplate on first - set at about 65 ºC. BUT, do not leave it unattended once switched on.
2. We use 15 mm diameter copper tubing (with a wooden handle) to make the wax rings on slides. We prefer to heat both the copper tubing and the wax; the copper tubing stood directly on the hot plate, next to the Pyrex dish containing paraffin wax (no more than 100 ml – too much makes messy slides).
3. Allow the wax to melt, but not burn –adjust the temperature of the hot plate as necessary. Do not heat the wax rapidly, it will denature, the temperature of the wax should be carefully controlled. If wax is over heated your slides may leak. It can take up to an hour for wax to melt, particularly if you have too much in the dish!
4. Slides and coverslips should be stored completely submerged in 80% alcohol (Industrial Methylated Spirits).
5. Prepare slides and cover-slips by drying with a tissue to ensure they are free from dust and grease. You may need to blow on them to remove final dust particles. Dust and grease can prevent the wax holding a slide and cover-slip together. Place the slides and cover-slips on a tissue and cover with a second tissue until they are required.

Making slides

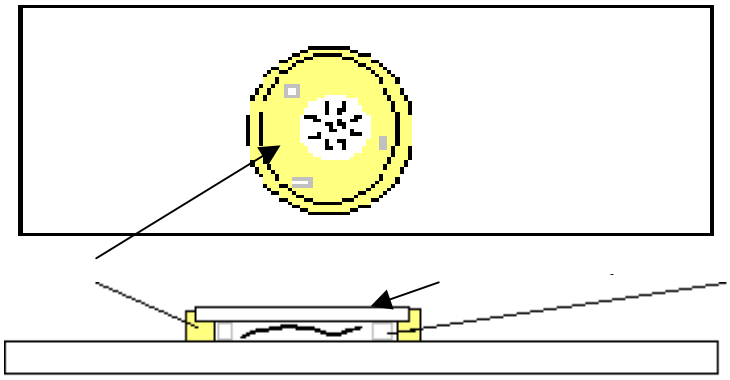
6. Stand the copper tubing in the wax.
7. Line up the clean slides, checking once more for dust.
8. The wax has completely melted when there are no signs of solid wax on the copper tubing. You need to practice until you are familiar with your equipment. Wax may appear melted, but solidify to quickly – it may need longer to heat through, but also test the temperature of the wax.
9. To apply the wax ring:
    i. Take the copper tubing by the handle and gently shake 4 – 5 times. This should remove excess wax.
    ii. Quickly make a wax ring in the centre of a slide by gently but firmly pressing the copper tubing flat onto the slide.
    iii. Rotating the copper tubing gently will help to even applying the wax.

10. We make all the wax rings required in one batch, but you may find it easier to complete each slide before making the next.
11. To mount specimens:
    i. Take the cavity block containing nemtodes from the dessicator but keep it covered to avoid rehydration of the specimens.
    ii. Place a small drop of anhydrous glycerol in to the centre of the wax ring on your first slide. *Anhydrous glycerol should be stored in a dessicator and when it is out for use the lid kept on whenever possible.*
    iii. Anhydrous glycerol readily absorbs water from the air - it is important to only put glycerol on to each slide as it is need and to work with it promptly
    iv. Check that there is no detritus in the glycerol under the microscope.
    v. With the watch-glass of nematodes under the microscope and the slide to one side on the stage, systematically pick nematodes from the watch-glass and place them into the centre of the glycerol on the slide.
    vi. To avoid bias in choice of animals mounted, it is best to work from one side of the watch glass to the other, removing all animals encountered. For example, start in the top left-hand corner move from left to right and work down the watch glass picking each animal encountered in turn.

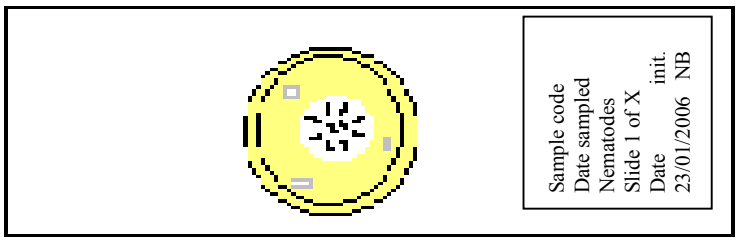




12. Mount approximately 10 animals per slide. Arrange them so that they are well spaced, and gently press each individual on to the slide so that it is not floating in the glycerol. This should help the nematodes stay in place when the slide is sealed.
13. If very large or small animals are encountered try to mount these together, it makes focusing at higher magnification much easier and clearer and will aid identification. In these instances, mount as many individuals per slide as is appropriate – 2 to 20 per slide!
14. With extremely large animals it may be that only one or two animals can be mounted per slide. In this instance it may be necessary to mount the nematodes on large slides with square cover-slips.
15. Once the nematodes are in the glycerol on the slide add 45 μm diameter glass beads. These should be positioned between the wax and the glycerol near to the wax in about 3 places. Only 3 or so beads are required in each location. Beads help to keep the slide level and prevent crushing of the animals*.
16. Next pick-up a clean cover-slip using the slide forceps (At this stage it may be necessary to blow on the cover-slip to remove any remaining fine particles of dust) and place it directly over the mounted nematodes, central to the wax ring.



17. Place the slide on the hot plate and allow the wax to re-melt, at least a few minutes. If air bubbles form, leave the slide on the hot plate for a few more moments, they should move out from under the cover-slip. If this does not happen, with the slide still on the hot plate gently tap the slide, this should start the movement of air bubbles. Try to remove as many air bubbles as possible – they hinder focusing and identification at high power.
18. Remove the slide to a safe place to cool and repeat with all material in the cavity block.
19. It is essential that the slides are fully labelled immediately after making, once they are cooled – this should limit the risk of mis-labelling later.
20. Remember to note how many animals are mounted on each slide. This is helps speed of identification and ensures that no specimens are accidentally missed.
21. Remember to note how many slides you have made for each sample. This is VERY important and helps ensures that no material is lost.
22. A pedantic but very helpful point –put the slide labels on the slides with text facing outwards – it is easier to read the labels when the slides are stored!



11. The watch glass should be thoroughly examined to ensure that no animals have been missed. When you are satisfied no specimens remain, wash the watch glass with detergent and dry it.
12. Ensure that the wax heater is turned off after use and is not left unattended whilst switched on.

* If you do not have glass beads, you can use 3 tiny pieces of aluminium foil. However, we have found that the companies selling glass bead sell them to industries in 25 kg bags (expensive) and are happy to send a small sample (which will last a nematologist for years) for free!

13.12 Sample processing log

SAMPLE PROCESSING LOG
MANUELA Meiofauna

Sample Code	
Extraction	
Water Decant	*Date* _____ *Initials* _____
Rose Bengal Added	*Tick* _____ *Initials* _____
Residue Archived	*Date* _____ *Initials* _____
Sample Splitting	
Splitting Required?	*Y / N (Delete as applicable) Initials* _____
METHOD	
Sample Split	*Date* _____ *Initials* _____
Sample Picking	
Name of Technician	*Name* _____ *Initials* _____
Start Date _____	Finish Date _____
Number of splits picked / counted	_____
Nematodes	
Number of nematodes picked to cavity block	_____
Nematode cavity block to dessicator	*Date* _____ *Initials* _____
Nematode slides mounted	*Date* _____ *Initials* _____
Nematode slides to storage for identification	*Location*_____ *Date* _____ *Initials* _____
Copepods	
Number of copepods picked to pots	_____
Copepods slides passed to	*Name*
	*Location*_____ *Date* _____ *Initials* _____

13.13 Nematode recording sheet

MANUELA Nematode Data

Sample Code	Microscope	ID'd by	Date	Sheet 1 of _____

No.	Slide	Track	Specimen (Species name)	D/A/M	No.	Slide	Track	Specimen (Species name)	D/A/M
1	1 of				26				
2					27				
3					28				
4					29				
5					30				
6					31				
7					32				
8					33				
9					34				
10					35				
11					36				
12					37				
13					38				
14					39				
15					40				
16					41				
17					42				
18					43				
19					44				
20					45				
21					46				
22					47				
23					48				
24					49				
25					50				

Notes
D/A/M = Dead /Alive/ Moribund?
Track = recorded from vernier scale (note whether up/down or left/right)

14 Appendix: Analysis of environmental samples

14.1 Particle size analysis

14.2 Analysis of chlorophyll a

14.3 Analysis of salinity

14.4 Analysis of permeability